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TITLE OF THE INVENTION

# A METHOD FOR ACQUIRING INFORMATION OF A BIOCHIP USING TIME OF FLIGHT SECONDARY ION MASS SPECTROMETRY AND AN APPARATUS FOR ACQUIRING INFORMATION FOR THE APPLICATION THEREOF

## BACKGROUND OF THE INVENTION

### Field of the Invention

**[0001]** The present invention relates to an imaging of ~~respective~~ a matrix disposed on a surface of a biochip, ~~that~~ which includes a substrate and a plurality of biological ~~related~~ materials disposed on a surface of the substrate in a matrix form, and also relates to an analysis of the components of ~~respective~~ the matrix.

### Description of the Related Art

**[0002]** A biochip, such as a DNA chip, protein chip and so on, which includes a substrate and various ~~probe~~ molecular probes disposed on a surface of the substrate in a matrix form, has been employed for the purposes of analyzing a genome or analyzing a generation of a gene. Further, it is expected that the result of the analysis ~~by using the~~ employing biochips provides a critical index for diagnosis of cancers, genetic diseases, life style-related diseases, infectious~~on~~ diseases and the like, prediction for

prognostics, or a decision ~~of~~ on treatment policy and so on.

**[0003]** Several methods for preparing biochips are known. ~~On describing the methods for preparing a DNA chip as examples, the e~~Exemplary methods for preparing a DNA chip may include: a method of consecutively synthesizing DNA probes directly onto a substrate by using photolithography (US Patent No. 5,405,783 and so on); or a method for supplying synthesized DNA or synthesized cDNA (complementary DNA) onto a substrate and ~~being bound~~ binding it thereto (US Patent No. 5,601,980, Japanese Patent Laid-Open No H11-187,900 (1999), an article ~~from~~ in "SCIENCE", Vol. 270, pp. 467 (1995) and so on).

**[0004]** In general, the biochip ~~are~~ is formed by using one of the two methods described above, and when the thus-formed biochip is used for the applications described above, it is critical to know quantities, i.e., densities, of biological ~~related~~ materials used for forming probes that are included in ~~respective matrix,~~ matrices for the purpose of ensuring the credibility of the analysis, i.e., the quantification or the reproducibility of the analysis. Further, it is also critical to know what type of matrix dimension (i.e., shape, size or condition) is provided to the matrix existing thereon (i.e., imaging)~~,~~ for the purpose of assuring the quantification-ability or the reproducibility of the analysis. In addition, as described later, if there is no physical address for indicating the

expected position of a ~~respective~~ matrix ~~to be located~~ on the substrate that is employed for forming chips, an additional problem may ~~be~~ occur~~red~~. More specifically, when the biochip is formed by using a method of supplying fine droplets of a probe solution thereto via the ink jet method, for example, an absence of the physical address thereon may lead to an unclear determination ~~on~~ of the position of the probe portion ~~where~~ when the analysis is ~~now~~ conducted on the biochip, depending on employed method. In such case, the detection means itself must also ~~function as~~ enable~~ing~~ a clear determination of the matrix position.

**[0005]** However, the probe on the biochip exists principally as a monolayer or less, and in general, the analysis of the biological ~~related~~ materials including the clear determination of the matrix position requires ~~the~~ highly sensitive surface analysis techniques.

**[0006]** One of the known highly sensitive surface analysis techniques that satisfies~~y~~ the aforementioned requirements may be a method of using stable isotope labeled probes. However, this~~e~~ method ~~contains~~ has various disadvantages from the viewpoint ~~in view~~ of ~~applying~~ general purpose usage.~~,~~ ~~that is,~~ Specifically the method requires ~~a~~ complicated labeling ~~method~~, ~~and the method requires~~ as well as special facilities and special equipments~~,~~ ~~since~~ because the employed isotope itself may be a source of a radioactive ~~pollution~~emission.

[0007] Another method may be ~~a method~~ that of labeling the probe with a fluorescent label, or alternatively, ~~a method~~ that of labeling a specific material that specifically binds to the probe with a fluorescent label and then binding it to the probe, which is known as a fluorescent-hybridization method for the DNA chip. However, ~~the~~ such a method also ~~contains~~ has various problems ~~against~~ with respect to achieving higher quantification-ability, such as a problem of the chemical stability of the fluorescent dye used for labeling, a problem of the fluorescent quenching, a problem of the nonspecific adsorption of the fluorescent dye onto the substrate surface, or additionally the problem of the quantification-ability (i.e., stability, reproducibility) of the specific binding-ability (i.e., hybridization), ~~and~~ ~~t~~Thus, there are a number of problems for quantitatively detecting the amount of the existing probe itself.

[0008] Other highly sensitive surface analysis methods that are capable of being employed for analyzing general detection objects include the ATR method that utilizes FT-IR ~~method~~ (Fourier Transform Infra Red Spectroscopy), XPS ~~method~~ (X-ray Photoelectron Spectroscopy) and so on. However, these methods do not involve sufficient sensitivity for the quantitative analysis of the probe ~~of the~~ on a biochip, i.e., a biological ~~related~~ material, or imaging thereof. In particular, when a general purpose glass is employed as a substrate for producing the biochip,

these methods are not available ~~methods~~, ~~since~~ because the absorption due to the glass substrate itself adversely affects the analysis results when the FT-IR (ATR) method is employed, for example, or ~~since the charge-up~~ because a charge-up occurred on the glass, which is an electrically insulating material, adversely affecting~~s~~ the analysis ~~results~~ when the XPS method is employed.

**[0009]** Yet another highly sensitive surface analysis method that is capable of being employed for analyzing biological related materials may be a DNA detection method utilizing the laser RIS (Resonance Ionization Spectroscopy) method, which is disclosed in United States Patent No. 5,821,060. In this method, the specimen surface is irradiated with laser or ion beams mentioned below, and the generated portion is irradiated with a laser beam having a wavelength that is equivalent to ionization energy of a specific element, so that the specific element is ionized and emitted from the specimen surface and the emitted ionized element is detected. Disclosed methods for releasing the element from the specimen surface may be a method utilizing a laser beam (laser ablation) or a method utilizing ions (ion sputtering). However, these methods have a technical limitation in ~~which~~ that only a limited number of elements ~~are possible to~~ can be detected.

**[0010]** Yet another highly sensitive surface analysis method may be dynamic SIMS (Secondary Ion Mass Spectrometry), in which an organic compound is decomposed

to smaller fragment ions or to particles during the process of generating a secondary ion. Thus, the amount of the information on the chemical structures obtained from the mass spectrum is not sufficient.~~, and t~~ Thus, the method is not generally suitable~~ for general purposes~~, ~~since~~ because the obtained information is not sufficient for the analysis of organic compounds such as, for example, nucleic acid-related materials having only ~~common~~ four common bases.

**[0011]** On the other hand, the time of flight secondary ion mass spectrometry (TOF-SIMS), which is also known as another technique of the secondary ion mass spectrometry, is an analysis method for investigating what types of atoms or molecules ~~are~~ exist~~ing~~ on the uppermost surface of a solid specimen.~~,~~ ~~and the~~This ~~method~~ has the following advantages: having ~~a detection~~ an ability ~~for~~ to detect~~ing~~ a trace amount of a component of $10^9$ atoms/$cm^2$ (equivalent to $1/10^5$ of ~~the~~ all atoms existing in one atomic layer of the uppermost surface); being applicable to both organic and inorganic compounds; being capable of detecting all types of elements and compounds that exist~~ing~~ on the surface; and being ~~available of~~ able to image~~ing~~ secondary ions from materials that are ~~existing~~ on the surface of the specimen.

**[0012]** Here, the principles of the time of flight secondary ion mass spectrometry will be described as follows.

**[0013]** At~~In~~ high vacuum ~~condition~~, a high speed pulsed ion beam (primary ion) irradiated to a surface of a solid specimen causes sputtering ~~phenomenon~~, in which a structural component~~s~~ of the surface ~~are~~ is emitted into the vacuum. Ions (secondary ions) having positive or negative charges generated during this process are accelerated into a mass spectrometer, where they are mass-analyzed by measuring the travel time from the specimen surface to a detector. In the sputtering process, various ions having a variety of masses are generated depending on the chemical components of the surface of the specimen, and the ions having a smaller mass fly faster and, on the contrary, ions having a larger mass fly slower, within a constant electrical field. Thus, detecting the time ~~taken~~ elapsed from the generation of the secondary ions to the arrival of the generated ions to the detector (i.e., time of flight) provides an analysis of the mass of the generated secondary ions.

**[0014]** On the other hand, in the dynamic-SIMS method, organic compounds are decomposed to small fragment ions or particles during the ionization process as stated above.~~, and thus~~ Thus, information on the chemical structure obtained from the mass spectrum, e.g., mass range, is limited. On the contrary, in the TOF-SIMS method, the structures of the organic compounds can be directly obtainable from the mass spectrum with a wide mass range, ~~since the extremely~~ because a much smaller amount of the

primary ions is necessary in the TOF-SIMS method, so that while the organic compounds are ionized, they ~~with~~ substantially ~~retaining~~ retain their chemical structure. In addition, the information on the uppermost layer (within a depth of several angstroms) of the object can be selectively obtained~~able~~ as only the secondary ions generated in the uppermost solid surface are emitted into the vacuum.

**[0015]** The TOF-SIMS apparatus that employs the principle of the measurements described above is generally classified ~~to~~ as a sector-type apparatus and a reflectron-type apparatus. One of the differences between these two types is ~~on~~ in the manner of electrically grounding of a holder that fixes an object to be analyzed. In the sector-type apparatus, the generated ions are led to the mass spectrometer by applying positive or negative voltage of several kV to the specimen-fixing holder~~, and on the contrary,~~. ~~in~~ In the reflectron-type apparatus, the specimen-fixing holder is grounded and the secondary ions are led to the mass spectrometer by applying positive or negative voltage of several kV to several-ten kV to an extracting electrode for the secondary ions.

**[0016]** The TOF-SIMS method often utilizes positive primary ions, and both positive secondary ions and negative secondary ions are generated regardless of the polarity of the utilized primary ions. Also, regardless of the polarity of the utilized primary ions, the amount of the

secondary electrons that are generated by irradiating the primary ions is greater than the primary ions ~~in~~ under the general measurement conditions, so that the surface potential tends to be positive.~~, and~~ ~~in~~ In turn, when the positive charge accumulates beyond a certain level (i.e., charge-up condition), the excessive positive charge may disturb the quantitative measurements. In considering the apparatus configurations in relation with the charge-up condition, the measurements of the negative secondary ions from the insulator material by using the sector-type apparatus can cause the highest positive-charge accumulation, ~~(~~because all of the generated secondary electrons are directed toward the extracting electrode for the (negative) secondary ions, ~~in which~~wherein the ~~extracting electrode is applied with the~~ above-mentioned positive voltage is applied to the extracting electrode.~~)~~

**[0017]** In order to neutralize the positive charge caused by the above-mentioned charge-up condition, both the sector-type apparatus and the reflectron-type apparatus may often be equipped with a pulse-type electron gun for neutralizing the charge. A s~~S~~pecific method for neutralizing the charge by using the pulse-type electron gun may include a step of applying the electron beam from the above-mentioned pulse-type electron gun onto the object to be analyzed for a constant duration irradiating primary ions (sub-nanosecond pulse to several nanosecond pulse) and before irradiating the primary ions for the next process of

generating secondary ions. Here, while the electron beam is ~~irradiating~~ irradiated by the pulse-type electron gun onto the object to be analyzed, the application of the voltage to the object holder (for the sector-type apparatus) or to the secondary ion extracting electrode (for the reflectron-type apparatus) is~~are~~ stopped, and the holder or the electrode ~~are~~ is grounded, respectively.

**[0018]** The above-mentioned method of neutralizing the charge often relieves (or compensates for) the ~~charged-up~~ accumulated positive charge, enabling the analysis of the insulator material. Here, when the negative secondary ions are measured for the insulator material by using the sector-type apparatus, the insulator is most-considerably and positively charged, and thus the margin of the charge-neutralization in this type of measurement is the narrowest. In ~~any way, in~~ order to prevent the ~~charging~~ charge-up, using the reflectron-type apparatus, in which the object holder is constantly electrically grounded ~~constantly~~, is (in general) more advantageous than using the sector-type apparatus. In particular, when the object to be analyzed has a lower electric conductivity (in other words, higher electric resistivity or a lower dielectric constant), e.g., glass and the like, a reflectron-type apparatus is more suitable for carrying out the quantitative measurements.

**[0019]** Regardless of ~~employing~~ whether a reflectron-type apparatus or a sector-type apparatus is employed, the TOF-

SIMS method is the analysis method of a considerably higher sensitivity~~,. so that t~~ Thise method enables the analysis of an object ~~to be analyzed of~~and is less ~~influential~~ influenced ~~with~~ by a ~~charging~~charge-up, e.g., oligonucleotide formed in a single molecular film level on a gold substrate having better electric conductivity. (Proceeding of the 12th International Conference on Secondary Ion Mass Spectrometry, 951 (1999)). Further, an evaluation conducted by the present inventors shows that, by conducting the process of preventing the charging-up, ~~the~~ biological ~~related~~ materials such as oligonucleotide bound to the substrate surface ~~of~~ with a higher dielectric constant, such as a glass substrate, can be in-situ analyzed by irradiating the primary ions at a spot ~~having a diameter of~~ several μm ~~level~~ in diameter when the analysis is conducted by an individual spot measurement.

**[0020]** However, the evaluation conducted by the present inventors also shows that when the two-dimensional secondary ion image was to be obtained by sequentially scanning the primary ion beam having a beam diameter of 5 μm in a constant direction, like the scanning line of a~~the~~ TV receiver (i.e., raster scanning), onto the substrate of a higher resistivity across a ~~certainly~~ wide area, e.g., the area ~~of~~ that is 500 μm x 500 μm, a good image was not obtained because of considerable influence of the ~~charging~~ charge-up.

## SUMMARY OF THE INVENTION

**[0021]** The present invention provides a solution for the aforementioned problems. The present invention provides a measurement method, which enables one to obtain a two-dimensional image with better quantitative-ability by suppressing the influence of the ~~charging-up,~~charge accumulation when the two-dimensional secondary ion image is obtained for a biological ~~-related~~ material fixed on a substrate having high resistivity by utilizing a TOF-SIMS method ~~in~~ over a ~~certainly~~ wide area.

**[0022]** The present inventors have actively ~~involved~~ investigated~~ions~~ ~~for~~ the above-mentioned problems, i.e., looked~~ing~~ for a solution for suppressing the influence of the charge~~ing~~-up when two-dimensional imaging is conducted via the TOF-SIMS method for a relatively large area of the portion of a biochip that includes a biological ~~-related~~ material formed on a substrate ~~of~~ having a relatively high resistivity~~,~~. ~~and~~ ~~t~~The present inventors have found ~~from results of our investigations provided~~ that a two-dimensional image having a considerably high positioning resolution-ability can be obtained by the procedure~~,~~ in which the pulsed primary ion beam is irradiated at a spot, and the pulse-wise spot-applications of the primary ion beam and the simultaneous detection of the secondary ion generated from the irradiated primary ion beam ~~are~~ proceeded along with a discontinuous scanning pattern, and eventually ~~the results of~~ these secondary ion measurements

results ~~is~~ are reconstructed into a two-dimensional image in line with the aforementioned discontinuous scanning pattern. Further, the present inventors have also confirmed that, when the pulsed primary ion beam is irradiated along with the aforementioned discrete pattern, the charge~~ing~~-up of some insufficiently charge-neutralized spots has dissipated until the detection of the secondary ion for the adjacent spots is conducted~~,~~. ~~and t~~ Therefore, the present invention has been ~~made~~ achieved on ~~the~~ this basis ~~of these knowledge~~.

**[0023]** That is, ~~a method for acquiring information from the biochip~~ according to the present invention, ~~may be~~ a method for acquiring information in relation to a biochip, which includes~~ing~~ a substrate and a plurality of biological-related materials disposed on a surface of the substrate, from the surface of the biochip using time of flight secondary ion mass spectrometry, includes~~ing~~ at least the steps of:

irradiating a pulsed primary ion beam on the surface of the biochip in a discontinuous pattern, the surface of the biochip having the biological-related material disposed thereon, and the primary ion beam having a spot size of an area that is a much smaller area than ~~an area~~ the one to be measured on the surface of the biochip;

conducting mass-analysis of secondary ions via time of flight, the secondary ion being generated by irradiating the pulsed primary ion beam; and

reconstructing analyzed results obtained by conducting the mass-analysis to form ~~a~~ two-dimensional distribution information on the basis of the pattern of the applying primary ion beam in a pulse manner.

**[0024]** Further, a method for analyzing components ~~of a~~on a biochip surface according to the present invention may be a method for analyzing components of a biological ~~-related~~ material disposed on a biochip, ~~in relation to the biochip~~ which includes a substrate and a plurality of biological ~~related~~ materials disposed on a surface of the substrate, from the surface of the biochip using time of flight secondary ion mass spectrometry, including at least the steps of:

irradiating a pulsed primary ion beam on the surface of the biochip in a discontinuous pattern, the surface of the biochip having the biological ~~-related~~ material disposed thereon, and the primary ion beam having a spot size area that is ~~of~~ much smaller ~~area~~ than an area to be measured on the surface of the biochip;

conducting mass-analysis of secondary ions via time of flight, the secondary ion being generated by irradiating the pulsed primary ion beam;

reconstructing analyzed results obtained by conducting the mass-analysis to form ~~a~~ two-dimensional distribution information on the basis of the pattern of the irradiating pulsed primary ion beam; and

conducting component-analysis of the biological ~~related~~ material of a necessary portion contained in the obtained two-dimensional image on the basis of the mass spectrum information of the necessary portion.

**[0025]** In addition, the present invention also provides an apparatus adopted to be used for acquiring information from the above-mentioned biochip surface, that is, an apparatus for acquiring information from the biochip surface according to the present invention ~~is~~ may be an apparatus for acquiring information in relation to a biochip including a substrate and a plurality of biological-related materials disposed on a surface of the substrate from the surface of the biochip using time of flight secondary ion mass spectrometry, including at least:

a device for irradiating a pulsed primary ion beam on the surface of the biochip in a discontinuous pattern, the surface of the biochip having the biological-related material disposed thereon, and the primary ion beam having a spot size of a much smaller area than an area to be measured on the surface of the biochip;

a device for conducting mass-analysis of secondary ions via time of flight, the secondary ion being generated by irradiating the pulsed primary ion beam; and

a device for reconstructing analyzed results obtained by conducting the mass-analysis to form ~~a~~ two-dimensional distribution information on the basis of the pattern of the irradiating pulsed primary ion beam.

[0026] Further objects, features and advantages of the present invention will become apparent from the following description of the preferred embodiments with reference to the attached drawings.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figs. 1-A, 1-B, 1-C and 1-D are images of the results of imaging according to Example 2, showing the imaging results obtained by reconstructing the data on the basis of $PO_2^-$ ion (Fig. 1-A); $PO_3^-$ ion (Fig. 1-B); $PO_2^-$ ion + $PO_3^-$ ion (Fig. 1-C); and $(thymine\text{-}H)^-$ ion (Fig. 1-D);

[0028] Fig. 2 is a graph showing the results of mass spectrum employed for obtaining the results of component analysis conducted in Example 2; and

[0029] Fig. 3 shows images prepared from the results of Example 8, and the images shown in the upper row are obtained by using $Ga^+$, and the images in the lower row are obtained by using $Au_3^+$.

## DETAILED DESCRIPTION OF THE INVENTION

[0030] The present invention will be fully described in detail as follows.

[0031] The method according to the present invention is characterized in irradiating pulsed primary ions on the basis of the discontinuous scanning pattern for acquiring the images via TOF-SIMS, not based on the above-mentioned raster scanning, and also characterized in carrying out the

imaging by reconstructing the respective mass analysis results obtained by respective discrete pulse-application on the basis of the pattern of the discontinuous pulse-application of the primary ion. The technique of scanning in the discontinuous scanning pattern enables imaging ~~the~~ of a relatively large area of the surface of the biochip that includes biological ~~-related~~ materials formed on the substrate having a relatively high resistivity.

**[0032]** The discontinuous scanning pattern may be any pattern that enables avoiding the influence of the charge~~ing~~-up~~,~~. ~~and~~ A typical discontinuous pattern may be a random pattern or a specifically programmed pattern. In such a case, although ~~an~~ overlapping of a~~n~~ unit (hereinafter called "pixel") being irradiated with primary ion beam (having same shape as the shape of primary ion beam) with the adjacent pixel may be permitted, the overlapping of the pixels is not preferable, ~~since the overlapping~~because it may cause ~~a~~ duplicated irradiation for an identical point in one scan~~ning~~, so that the obtained data does not reflect the actual value. Thus, if a random number is employed by the computer for generating ~~the~~ a random scanning pattern ~~of the scanning~~, the employed random number may preferably be one that is capable of providing a uniform ~~probability of the generation~~ scan across the area being irradiated. Also, a programmed specific pattern described above may optionally be used, if necessary. The programmed specific pattern described above

may preferably have discrete scan path tracks, each of which is sufficiently discrete or separated to avoid the charge~~ing~~-up problem. If the scan path tracks of the programmed specific pattern are sufficiently discrete, an effect equivalent to the one obtained by employing the random scanning can be expected by employing the programmed specific pattern. However, if the intervals between the discrete scan path tracks are short, or more specifically, for example, if the irradiation is carried out onto alternate pixels, or, in other words, if the irradiated pixels are relatively closely disposed, the influence of the charge~~ing~~-up cannot sufficiently be avoided. Thus, when the above-mentioned "programmed specific pattern" is employed, the scan path tracks of the pattern may preferably be designed to be sufficiently discrete.

**[0033]** When an image is formed by using a mass spectrum of the thus-obtained respective pixels, reconstructing ~~of~~ the data in the order of the measurements of the respective pixels may not provide ~~the~~ a suitable image that appropriately reflects the actual condition, ~~since~~ because the scanning of the primary ion beam is carried out ~~with~~ in the discontinuous pattern, i.e., random pattern, specifically programmed pattern and so on. In such a case, the present invention provides ~~the~~ a suitable image that appropriately reflects the actual conditions, by storing the irradiation pattern of the primary ion beam and

reconstructing the obtained data on the basis of the stored irradiation pattern.

**[0034]** The combination of the discontinuous application of the primary ion beam and the reconstruction~~ing~~ of the obtained data according to the present invention is considerably advantageous in the measurement using ~~the~~ a substrate having a high~~er~~ resistivity in which the measurement is considerably influenced by the charge~~ing~~-up~~,~~ . ~~and on~~On the other hand, the combination according to the present invention may not be fully advantageous in reality in the measurement using the substrate having a lower resistivity in which ~~the~~ suitable imaging can be carried out by using ~~the~~ ordinary raster scanning, ~~since~~ because the combination of the discontinuous scanning and the reconstruction~~ing~~ of the data requires a longer period of time to~~for~~ carry~~ing~~ out ~~the reconstructing of the data~~ than the ordinary raster scanning. In order to fully provide the advantages ~~there~~of the invention, the scanning technique may be selected depending on the resistivity of the substrate to be used. For example, the range of the resistivity of the materials for the substrate, in which the discontinuous scanning is considerably advantageous, is a volumetric resistivity of not less than $10^{10}$ ohm·cm (300K).

**[0035]** The volumetric resistivity of the substrate being preferably used for the substrate of the biochip may ~~be~~ not be less than $10^{10}$ ohm·cm (300K), and such a substrate is the

most suitable for applying the imaging method ~~of imaging~~ according to the present invention.

**[0036]** The species of the primary ion for the use in the present invention may preferably be a gallium ion ($Ga^+$) or a cesium ion ($Ce^+$), and, optionally, an Au ion ($Au^+$) and the like, in view of ionization efficiency, mass analysis resolution and so on. Here, the Au ion is more preferably used, because it~~of~~ provides~~ing~~ the mass analysis with a considerably higher sensitivity. In such a case, the available ion is not limited to the Au ion~~,~~. ~~but~~ An $Au_2$ ion and an $Au_3$ ion may be also used~~,~~. ~~and t~~The sensitivity of the measurement often increases by selecting the Au ion~~,~~ A greater increase is achieved ~~much increases~~ by selecting the $Au_2$ ion ($Au_2^+$). ~~A and~~ much greater ~~more~~ increase~~s~~ is achieved by selecting the $Au_3$ ion ($Au_3^+$), thus presenting more preferable measurements.

**[0037]** When the imaging is carried out by using TOF-SIMS, the measurement conditions of mass analysis resolution, area for analysis and time for analysis are not uniquely determined, ~~since~~ because the conditions are closely and mutually related to pulse frequency of the primary ion beam, energy of the primary ion beam, pulse width of the primary ion beam, and the data handling ability of the computer employed for ~~using~~ the image processing. However, each ~~value~~ of these conditions should be within a range for enabling the analysis.

**[0038]** In view of the availability of the analysis, the pulse frequency of the primary ion beam used in the present invention may preferably be in the range from 1 kHz to 50 kHz, the energy of the primary ion beam may preferably be in the range from 12 keV to 25 keV, and the pulse width of the primary ion beam may preferably be from 0.5 ns to 10 ns.

**[0039]** In order to improve the measurement accuracy, the measurement should be completed in a short period of time (an order of several ~~--~~tens of seconds to several ~~-~~tens of minutes) while maintaining the high mass resolution~~,~~. For ~~and for~~ this reason, the measurement may preferably be carried out without using a highly-focused primary ion beam~~, for the purpose of~~ to complete~~ing~~ the measurement in a short period of time. More specifically, it is not necessary to highly focus the aperture diameter of the primary ion beam ~~is not necessary to be highly focused~~ to a sub-micron level by a relatively complicated operation.~~,~~ ~~but~~ It may preferably be focused ~~to~~ at the level ranging from 1 μm to 10 μm by a relatively simple operation. This ~~range of the~~ diameter range is preferable, ~~in~~ considering that the size of the respective matrix (also called "dot" or "spot") on the biochip to be analyzed according to the present invention normally has a circular shape having a diameter ~~of~~ from 10 μm to 100 μm, or a rectangular shape ~~having a dimension of~~ that ranges from 10 μm x 10 μm to 100 μm x 100 μm.

**[0040]** The area for scanning is not uniquely determined, ~~since the area of scanning~~because it is related to other factors as mentioned above~~,~~ However, ~~but~~ preferably, this area has a circular shape having a diameter within a range from 50 μm to 500 μm, or ~~the~~ a rectangular shape ~~having a dimension within a~~ that ranges from 50 μm x 50 μm to 500 μm x 500 μm.

**[0041]** The number of the irradiating primary ion beams, i.e., the pixels, in one specific scanning process depends on the size of scanning area, the diameter of the primary ion beam, the level of the overlapping of the pixels, or the frequency of the primary ion beam or the scanning time for one scan~~ning, and the~~ ~~t~~These conditions automatically determine the number of the pixels composing the secondary ion image. In this sense, the secondary ion image may be composed of pixels within a range from 56 x 56 pixels to 1024 x 1024 pixels.

**[0042]** The outer size of a generally used biochip may be, for example, 1 cm x 1 cm, 1 inch x 1 inch (25.4 cm x 25.4 cm) or slide glass size (e.g., 26 mm x 76 mm), and the matrix may be disposed within this size. The sizes of the scanning areas illustrated above are not sufficiently wide for scanning across such~~these~~ size~~ds~~ ~~of the~~ biochip ~~for the~~ to image~~ing~~ ~~of~~ the entire surface thereof. In such a case, a process of positional scanning (in general, called "stage scanning", as a stage having a substrate thereon is scanned in this scanning process) of the substrate may be

optionally employed in addition to the primary ion beam ~~scanning~~ to scan a wider area of the surface, as required. In this case, a longer period of time for analysis is required if a wider area is scanned. However, since the matrix does not usually cover ~~across~~ the entire surface of the biochip, the necessary area for the analysis may be selected depending on the requirement, and the scanning area may preferably be ~~a~~ circular ~~shape having~~with a diameter of 1 mm or greater or a rectangular shape of a dimension of 1 mm x 1 mm or ~~broader~~larger, or more preferably, ~~a~~ circular ~~shape having~~with a diameter within a range from 10 mm to 30 mm.

**[0043]** As described above, the main feature of the present invention is the imaging of the biochip via TOF-SIMS. ~~In the reverse view thereof,~~From a different perspective, the imaging of the present invention is carried out on the basis of the mass data of the fragments, which can be detected, measured and analyzed by using TOF-SIMS. ~~In other view thereof~~From yet another perspective, the mass spectrum data can be principally extracted from the portion (or the pixel) in which the mass data of the biochip for imaging is detected. The present invention includes the component analysis of the portions in which the imaging is carried out and the positions thereof are specified. The imaging of the specified portions of the actually prepared biochip via this method enables the determination of the

positions and the shapes, and the component analysis of the positions.

**[0044]** The biological ~~-related~~ material disposed on the biochip, which is imaged or component-analyzed according to the present invention, is not particularly limited and may be any material as long as the material can be imaged or component-analyzed according to the TOF-SIMS method of the present invention~~,~~. ~~and a~~ According to the evaluation of the present inventors, nucleic acids and proteins are preferable for ~~being~~ the analysis~~zed~~. ~~The e~~Examples of the nucleic acids may include DNA such as oligodeoxynucleotides, polydeoxynucleotides, cDNA (complementary DNA) and so on, RNA, such as mRNA (messenger RNA), tRNA (transfer RNA), rRNA (ribosomal RNA) and so on, and nucleic acid analogues being typically represented by peptide nucleic acid (PNA), the molecular bone of which comprises peptides. Examples of the proteins may include oligopeptides, polypeptides, enzymes, antibodies and so on.

**[0045]** The ~~existing form of the~~ biological ~~-related~~ material on the substrate may be in any form~~,~~. However, it is ~~-but may~~ preferably ~~be a form of being~~ covalently ~~-~~ bonded ~~with~~ to the substrate surface, in view of the form of the use of the biochip (for example, the form of the hybridization in the case of the DNA chip) and the stability of, for example, the level of ionization during the analysis using TOF-SIMS method. Various methods are known for forming the covalent ~~-~~bond ~~of~~ between the

biological ~~-related~~ material ~~with~~ and the substrate surface, and ~~the~~ a suitable method can be selected form ~~these~~ known methods. An example of the method of forming the covalent bond is disclosed in the Japanese Patent Laid-Open No. H11-187,900 (1999).

**[0046]** Also, methods for sequentially synthesizing the nucleic acids and proteins on the solid phase materials are known for one form of forming the covalent bond, and these methods can be employed for preparing the biochip that is the object of the method according to the present invention.

**[0047]** Further, the method of ~~covalent~~ covalently bonding the biological ~~-related~~ material ~~with~~ to the substrate may also include ~~the~~ a method of covalently ~~-~~bonding a first functional group included in the biological ~~-related~~ material, e.g., a nucleic acid or a protein, with a second functional group bonded to the surface of the substrate~~,~~ by supplying the biological ~~-related~~ material onto the substrate, ~~in which~~ wherein the second functional group is capable of reacting with the first functional group to form the covalent bond therebetween. The method of supplying the biological ~~-related~~ material onto the substrate for employing in the present invention may include the ink-jet method typically including the known piezo-jet method and the thermal jet method. ~~The~~ Japanese Patent Laid-Open No. H11-187,900 (1999) also discloses ~~the~~ a method of supplying a DNA probe onto ~~the~~ a substrate by the thermal jet method.

**[0048]** It is necessary to detect the fragment ions that ~~is~~ are specific to the above-mentioned biological ~~-related~~ materials as secondary ions~~, for~~ in order to carry~~ing~~ out the imaging and the component analysis of the biochip via the TOF-SIMS method~~, .~~ ~~and the~~ The fragment ion may be any ion~~s~~, as long as ~~the~~ it ~~ion~~ is specific to the biological ~~-related~~ material and is capable of being detected by the TOF-SIMS method.

**[0049]** The non-limiting examples of the biological ~~-related~~ materials and ~~the~~ specific fragment ions ~~will be~~are described ~~in the followings~~ below.

**[0050]** When the biological ~~-related~~ material is ~~the~~ a nucleic acid, ~~the material~~ it must have the backbone consisting of diester phosphates~~, and~~. ~~t~~Therefore, the fragment ions of the nucleic acid may include P-, PO-, PO2- and PO3-, which are the fragment ions of the above-mentioned backbone of diester phosphate, and these ions are capable of being detected via the TOF-SIMS method.

**[0051]** Further, when the nucleic acid is DNA, the material should include ~~four bases of~~ adenine, thymine, guanine and cytosine, and thymine is replaced with uracil in the case of RNA. Also, PNA, an exemplary nucleic acid analogue, should include ~~four bases of~~ adenine, thymine, guanine and cytosine. Thus, fragment ions of these bases, i.e., (adenine-H)-, (thymine-H)-, (guanine-H)-, (cytosine-H)- and (uracil-H)- can be employed for the secondary ions.

**[0052]** PNA also has a backbone that constitutes peptides~~,~~ . ~~and t~~ Thus, fragment ions of peptides, such as CNO- ion or CN- ion, can be employed for the detection via the TOF-SIMS method.

**[0053]** When the biological ~~related~~ material to be detected is a protein, the fragment ions of the peptides can be employed, because ~~since~~ the backbone of the protein contains~~stitutes~~ peptides, as in the case of PNA. In addition, fragment ions derived by the residual group of each amino acid can also be employed. Here, the efficiency of the detection for proteins is generally lower than the efficiency for nucleic acids, ~~since~~ because the mass spectrum intensity of one species derived by one amino acid of protein, which consists of more than 20 types of amino acids, is lower than the mass spectrum intensity of one species derived by one base of nucleic acids, such as DNA, RNA and PNA, which consists of four bases.

**[0054]** In the method for acquiring information, a TOF-SIMS apparatus for the use in performing two-dimensional imaging and component analysis may be any type of TOF-SIMS apparatus, as long as the apparatus is capable of performing detection, two-dimensional imaging and composition analysis. Here, the reflectron type apparatus, in which the holder for fixing the substrate is usually grounded, is preferably employed, ~~in view of the purposes for~~due to the need to effectively reduce~~ing~~ the influence of the charge~~ing~~-up that occurs~~red~~ on the substrate during

the handling of the insulator material, as stated ~~before~~above.

EXAMPLES

**[0055]** The present invention will be described more specifically~~,~~ by illustrating examples.

~~(EXAMPLE~~ Example 1~~)~~ Preparation of a nucleic acid probe chip by using a dT40 probe

**[0056]** A nucleic acid probe was prepared by using quartz glass, similarly as in the method described in the Japanese Patent Laid-Open No. H11-187,900 (1999).

(1) Washing of the substrate

**[0057]** A 25.4 mm x 25.4 mm synthesized quartz substrate ~~having a dimension of 25.4 mm x 25.4~~ mm was ~~disposed in~~ placed on a rack~~,~~ and ~~the substrate was~~ immersed in a ~~detergent~~ solution ~~that~~ containing~~s~~ a detergent for ultrasonic washing (GPIII, commercially available from BRANSON) diluted to 10% with pure water for one night. Then, the substrate was ultrasonic-washed in the detergent solution for 20 minutes~~,~~ and ~~after that~~ then ~~substrate was~~ washed with water to remove the detergent. After being rinsed with pure water, the substrate was further ultrasonic-washed within a container containing pure water for 20 minutes. Next, the substrate was immersed in an aqueous solution of 1N sodium hydroxide that was pre-heated to 80°C for 10 minutes. Sequentially, the substrate was

washed with water and further washed with pure water, and the washed substrate was transferred for further ~~to the next~~ processing without conducting a drying process.

(2) Surface treatment

**[0058]** An aqueous solution of 1%wt. of N-ß-(aminoethyl)-g-aminopropyltrimethoxysilane, KBM603 (commercially available from SHIN-ETSU CHEMICAL IND. CO. LTD.), which is a silane coupling agent having amino acids bonded thereto, was stirred at room temperature for 2 hours to achieve a hydrolysis of the methoxy group contained in the molecule~~ar~~ of the silane compound. The ~~washed~~ substrate that was washed ~~in the process~~ as described in the above section (1) was then immersed into the aqueous solution of the silane coupling agent for 1 hour, and after that the substrate was washed with pure water, and ~~the~~ both sides of the substrate ~~was~~ were dried by ~~being~~ blowing~~n~~ ~~with~~ nitrogen gas ~~to the both sides~~thereon. Next, the substrate was baked in an oven that was heated to 120°C, for 1 hour, and thereby, amino acids were eventually introduced onto the surface of the substrate.

**[0059]** Next, 2.7 mg of N-(Maleimidocaproyloxy)succinimide (commercially available from DOJINDO LABORATORIES, hereinafter called "EMCS") was dissolved into a solution of 1:1 (by volumetric ratio) of dimethyl sulfoxide (DMSO)/ ethanol to prepare a solution having a concentration of 0.3 mg/ml. The substrate, which had been treated via the

silane-coupling treatment, was immersed in the EMCS solution at room temperature for 2 hours to react the amino group, which is introduced to the substrate surface via the silane coupling treatment, with the succinimide group of EMCS. The reaction introduced a maleimide group derived from EMCS present ~~existing~~ on the substrate surface. The substrate was then picked up from the EMCS solution, was washed with the aforementioned DMSO/ethanol solution, was washed with ethanol, and then was dried by ~~being~~ blowing~~n~~ ~~with~~ nitrogen gas thereon.

(3) Synthesis of probe DNA

**[0060]** Single strand nucleic acid of base sequence No. 1 (40mer of dT) was synthesized~~,~~ by ~~ordering~~ a DNA synthesis company (BEX CO. LTD.). Sulfanilic group (SH) was introduced to the 5' end of the single strand DNA of the base sequence No. 1~~,~~ by using a thiol modifier (available from GLENN RESEARCH CENTER). After the DNA synthesis ~~of DNA~~, the deprotecting and the recovering of DNA were carried out according to the ordinary methods, ~~and~~ DNA was purified by using HPLC. The ~~series of the~~ processing from the synthesis to the purification was conducted by the aforementioned DNA synthesis company.

**[0061]** Sequence No. 1

5'HS-$(CH_2)_6$-O-$PO_2$-O-TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT 3'

(4) DNA discharge by using a thermal jet printer and binding of DNA to the substrate

**[0062]** The single-stranded DNA described in the above section (3) was dissolved into an solution, which contained 7.5%wt. of glycerin, 7.5%wt. of urea, 7.5%wt. of thioglycol, and 1%wt. of acetylene alcohol (under the product name of "ACETYLENOL EH", commercially available from KAWAKEN FINE CHEMICAL CO., LTD.), to obtain an eventual concentration of 8 μm.

**[0063]** Meanwhile, a printer head ("BC-50", commercially available from CANON CO. LTD.) for a bubble jet printer ("BJF-850", commercially available from CANON CO. LTD.), which employs a bubble jet method that is one of the thermal jet methods, was altered so that the altered printer head was capable of discharging several-hundred ml of the solution. The altered printer head was mounted to a discharge drawing device, which was also altered so as to be capable of discharging the solution onto the flat quartz substrate. Several-hundred ml of the above-mentioned DNA solution was transferred into an altered tank of the printer head, and the EMCS-treated substrate was mounted to the discharge drawing device to carry out a spotting operation onto the EMCS-treated surface of the substrate. Here, the discharge rate during the spotting operation was 4 pl/droplet, the area of the spotting operation was 10 mm x 10 mm, and the spotting was carried out at 200 dpi for that area, i.e., the discharge was performed at a pitch of

127 μm. ~~In this~~ Under these conditions, the diameter of the spotted dot was approximately 50 μm.

**[0064]** After completing the spotting operation, the substrate was left in a humidifier chamber for 30 minutes so that the maleimide group of the substrate surface ~~was~~ would reacted with the sulfanilic group (SH) of the 5' end of the nucleic acid probe to fix the DNA probe thereon. Then, the substrate was washed with ~~pure water,~~ and stored in ~~the~~ pure water. The obtained DNA-combined substrate (DNA chip) was dried by being blown on with nitrogen gas, and was stored in a vacuum ~~decicator~~ desiccator to be further dried, just before conducting the analysis via TOF-SIMS.

~~(~~Example 2~~)~~ Imaging and composition analysis via TOF-SIMS

(1) Operations

**[0065]** ~~Operations of t~~The imaging and the composition analysis for the DNA chip prepared in the above-mentioned Example 1 were carried out by using a "TOF-SIMS IV" apparatus, which is commercially available from ION TOF CO. LTD.

**[0066]** The apparatus and conditions used in this operation are listed below.

**[0067]** <primary ion>

primary ion beam: 25 kV, $Ga^+$, 0.6 pA (pulse current), random scan mode;

pulse frequency of the primary ion beam: 2.5 kHz (400 μsec./shot);
pulse width of the primary ion beam: 1 ns; and
beam diameter of the primary ion beam: 5 μm.
<secondary ion: imaging was carried out by reconstructing the obtained data according to the application pattern of the primary ion beam>
detection mode for secondary ion: negative;
area for the measurement: 300 μm x 300 μm;
number of pixel in the secondary ion image: 128 x 128 pixels; and
number of integrating operation: 256.

(2) Measurement results

**[0068]** Fig. 1 shows the results of the imaging for the typical ion species from the data obtained by analyzing the DNA chip prepared in the Example 1 using the "TOF-SIMS IV" apparatus under the conditions described above. Fig. 1-A and Fig. 1-B represent the results of imaging of the $PO_2^-$ ion and the $PO_3^-$ ion, respectively, both of which are the fragment ions of DNA phosphate backbones. As can be seen from these two-dimensional images, it was confirmed that DNA ~~existed~~ was present on the DNA chip in ~~a shape of~~ spotted form deposited by using a bubble jet device (i.e., a substantially circular shape having a diameter of about 50 mm, and the pitch between the dots being about 125 μm). It is also possible to obtain a two-dimensional image by

using the sum of the $PO_2^-$ ion and the $PO_3^-$ ion, as shown in Fig. 1-C, as well as the imaging of one fragment ion species.

**[0069]** It is also possible to conduct ~~an~~ imaging by using a $C_5H_5N_2O_2^-$ ion, ~~that~~ which is the fragment ion derived from the nucleic acid base, for example, as shown in Fig. 1-D, as well as one using the fragment ion of a phosphate backbone. Since the probe DNA used in the present example was a homo-oligomer of thymidylic acid, the detected fragment ion derived from the nucleic acid base was only the $C_5H_5N_2O_2^-$ ion, i.e., $(thymine\text{-}H)^-$ ion.

**[0070]** Fig. 2 shows mass spectrum profiles for the inner portion and the outer portion of a dot included in the obtained images concerning the typical secondary ions. For example, if the fragment ion is a $SiH_3$ ion, the ~~existence~~ presence of the ion is detected equally in either ~~of~~ the inner portion ~~and~~ or the outer portion of the dot, ~~since~~ because the $SiH_3$ ion is not specific to the DNA existing inside the dot. ~~On~~ To the contrary, if the fragment ion is $O2^-$, $P^-$, $PO^-$, $PO_2^-$, $PO_3^-$, $CNO^-$ (derived from the nucleic acid base) and $C_5H_5N_2O_2^-$, which are specific to the DNA existing inside the dot, or, in other words, ~~which~~ if the probability of the ion existing inside the dot is higher than the probability of existing outside the dot, the intensity of the detected ion strength for these ions ~~are~~ is stronger inside the dot than outside the dot. As seen in the results shown in Fig. 2, the use of the present

invention enables the component analysis of the portion, the position of which is determined, by conducting the two-dimensional imaging via the mass spectroscopy.

~~(~~Example 3~~)~~ preparation of a nucleic acid probe array by employing 50mer probe containing mixed four types of nucleic acid bases, imaging and component analysis thereof

(1) Preparation of DNA chip

**[0071]** DNA chip was prepared with DNA of the following base sequence No. 2, in the procedure identical to the procedure described in ~~the~~ Example 1.

**[0072]** Sequence No. 2

5'HS-$(CH_2)_6$-O-$PO_2$-O-TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG 3'

(2) Imaging and composition analysis via TOF-SIMS

**[0073]** Imaging and composition analysis for the DNA chip comprising DNA of the above-identified sequence No. 2 were conducted via the method and conditions identical to ~~that~~ these described in ~~the~~ Example 2.

**[0074]** The results ~~of the present Example~~ show that the imaging and the component analysis by the respective fragment ions of $(adenine-H)^-$, $(guanine-H)^-$ and $(cytosine-H)^-$ can be conducted, as well as the imaging and the component analysis for the fragment ions for the phosphate backbone and the fragment ions, such as $(thymine-H)^-$ described in ~~the~~ Example 2.

~~(~~Example 4~~)~~ Preparation of RNA chip, imaging and component analysis thereof

(1) Preparation of RNA chip

**[0075]** RNA chip was prepared with RNA (U20) of the following base sequence No. 3~~,~~ ~~in the~~ using a procedure that is identical to the ~~procedure~~ one described in ~~the~~ Example 1, except that all the preparation processes were carried out ~~under the condition of being~~ free of RNase that is an RNA decomposition enzyme.

**[0076]** Sequence No. 3

5'HS-$(CH_2)_6$-O-$PO_2$-O-UUUUUUUUUU UUUUUUUUUU 3'

(2) Imaging and composition analysis via TOF-SIMS

**[0077]** Imaging and composition analysis for the RNA chip comprising RNA of the above-identified sequence No. 3 were conducted via the method and conditions identical to ~~that~~ those described in ~~the~~ Example 2. Here, the RNA chip substrate was maintained ~~to be in the condition of~~ RNase free just until the TOF-SIMS analysis was started.

**[0078]** The results of the present Example show that the imaging and the component analysis by the fragment ion of (uracil-H)- can be conducted, as well as the imaging and the component analysis for the phosphate backbone-derived the fragment ions in ~~the~~ Example 2.

~~(~~Example 5~~)~~ Preparation of PNA chip, imaging and component analysis thereof

(1) Preparation of PNA chip

**[0079]** PNA having the base sequence identical to the base sequence of the DNA probe prepared in the Example 3 (referred to as Sequence No. 2') was synthesized~~,~~ by ~~ordering~~ a DNA synthesis company (BEX CO. LTD.). Here, cysteine, one of the amino acids, was bonded to the N end (corresponding to the 5' end of nucleic acid) via a linker described below. Since cysteine contains a (SH-) group in the branch, PNA ~~is possible to~~ can bind with the maleimide group ~~existing~~ present on the quartz substrate after its surface is treated.

**[0080]** The PNA chip was prepared with PNA of ~~the~~ sequence No. 2'~~,~~ ~~in the~~ using a procedure identical to ~~the procedure described~~ in ~~the~~ Example 1.

**[0081]** Sequence No. 2'

NCys-NH-$(CH_2)_2$-O-$(CH_2)_2$-O-$CH_2$CONH-TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG

(2) Imaging and composition analysis via TOF-SIMS

**[0082]** Imaging and composition analysis for the PNA chip comprising PNA of the above-identified sequence No. 2' were conducted via the method and conditions identical to ~~that~~ those described in ~~the~~ Example 2.

**[0083]** The results ~~of the present Example~~ show that the imaging and the component analysis by the respective fragment ions of $(adenine\text{-}H)^-$, $(thymine\text{-}H)^-$, $(guanine\text{-}H)^-$ and $(cytosine\text{-}H)^-$, derived from four bases that constitute~~s~~ PNA, can be conducted. Here, since PNA has no phosphate backbone, no fragment ion derived from the phosphate backbone was detected. ~~On~~ To the contrary, the fragment ions derived from the peptide bonds ~~contained~~ in the backbone of PNA, for example, $CNO^-$ ions and $CN^-$ ions, were detected.

~~(~~Example 6~~)~~ Preparation of protein chip, imaging and component analysis thereof

(1) Preparation of protein chip

**[0084]** A ~~P~~protein chip was prepared by fixing a protein ~~to~~ on a quartz substrate surface ~~in a different~~ using a method different from the methods for preparing synthesized nucleic acid probes described in Examples 1-5. ~~, and m~~More specifically, bovine serum albumin (BSA: commercially available from SIGMA ALDRICH JAPAN) was used. Here, BSA contains a cysteine residual group. ~~, and thus~~Thus, the protein was bound to the substrate surface via the reaction of SH- of cysteine and the maleimide group on the substrate surface.

**[0085]** Spotting ~~operation~~ of a protein solution was carried out as in ~~the~~ Example 1 to prepare the protein chip. Here, the conditions, such as the solvent ~~condition~~

and the BSA concentration during the discharging process of the BSA via the bubble jet, were ~~optimistically~~ accordingly adjusted.

(2) Imaging and composition analysis via TOF-SIMS

**[0086]** Imaging and composition analysis for the protein chip comprising the above-identified BSA fixed thereto were conducted via the method and conditions identical to ~~that~~ those described in ~~the~~ Example 2, except that the detection mode for the secondary ion was selected to be positive.

**[0087]** The results ~~of the present Example~~ show that the imaging and the component analysis by ~~the~~ several fragment ions of residual groups of amino acids can be conducted. Typical secondary ion species were: $C_4H_8N^+$ and $C_4H_6N^+$, which ~~that~~ are considered to be fragment ions derived by proline (Pro), $CH_3N^+$, $C_2H_7N_3^+$, $C_4H_{10}N_3^+$, $C_4H_{11}N_3^+$ and $C_5H_8N_3^+$, ~~that~~ which are considered to be fragment ions derived by an arginine (Arg) residual group; and $C_9H_8N^+$, $C_{10}H_{11}N^+$ and $C_{11}H_8NO^+$, which~~that~~ are considered to be fragment ions derived by a tryptophan (Trp) residual group. Further, $C_2H_6NS^+$ and $CHS^+$, ~~that~~ which are considered to be fragment ions derived by a cysteine (Cys) residual group, were also detected. As can be seen from the results described above, the detection of the above-mentioned fragment ions, which are considered to be derived by amino acid residual groups, enables the imaging of the protein disposed on the insulator substrate surface. When the protein having characteristic amino

residual groups is detected, an image equivalent to a two-dimensional distribution of the protein can be created by detecting the above-mentioned fragment ions. Further, a combination of the image analysis and numerical analysis for an image created by the respective above-mentioned fragment ions, which are considered to be derived by respective amino acid residual groups (e.g., digitalization of the amount of the amino acids contained in the protein ~~are~~ is conducted for a plurality of proteins is carried out and then the resultant digitalized data are correlated with the intensity of the above-mentioned fragment ions (i.e., image intensity)), can be carried out to obtain images (a two-dimensional distribution image) of respective proteins.

~~(~~Example 7~~)~~

**[0088]** Imaging and composition analysis for the DNA chip prepared in ~~the~~ Example 1 were conducted via the method and conditions identical to that described in ~~the~~ Example 2, except that the employed primary ion was $Au^+$. The results ~~of the present Example~~ show that the mass spectrum for the respective ions detected in Example 2 can be obtained with double-digit higher sensitivity and ~~the~~ better imaging on the basis of the mass spectrum with higher sensitivity ~~can be obtained~~.

~~(~~Example 8~~)~~ ~~p~~Preparation of a nucleic acid probe array by employing 13mer probe containing mixed four types of

nucleic acid bases, imaging and component analysis thereof by using TOF-SIMS method with the primary ion species of $Ga^+$ and $Au_3^+$.

(1) Preparation of DNA chip

**[0089]** A DNA chip was prepared with DNA of the following sequence No. 4~~, in the~~ using a procedure identical to ~~the procedure~~ that described in ~~the~~ Example 1.

**[0090]** Sequence No. 4

5'HS-$(CH_2)_6$-O-$PO_2$-O- ACTGGCCGTC GTTTTACA 3'

(2) Imaging and composition analysis via TOF-SIMS

**[0091]** Imaging and composition analysis for the DNA chip comprising DNA having the above-identified sequence No. 4 were conducted by using $Ga^+$ and $Au_3^+$ for primary ions (apparatus employed for the present Examples was "TOF-SIMS IV" commercially available from ION TOF CO. LTD). The conditions for measurements are listed below.

**[0092]** Case of using $Ga^+$ for primary ion species:

<primary ion>

primary ion beam: 25 kV, $Ga^+$, 0.6 pA (pulse current), random scan mode;

pulse frequency of the primary ion beam: 2.5 kHz (400 $\mu$sec./shot);

pulse width of the primary ion beam: approximately 1 ns; and

beam diameter of the primary ion beam: 5 $\mu$m.

<secondary ion: imaging was carried out by reconstructing the obtained data according to the application pattern of the primary ion beam

detection mode for secondary ion: negative;

area for the measurement: 300 μm x 300 μm;

number of pixel in the secondary ion image: 128 x 128 pixels; and

number of integrating operation: 256.

**[0093]** Case of using $Au_3^+$ for primary ion species:

<primary ion>

primary ion beam: 25 kV, $Au_3^+$, 0.07 pA (pulse current), random scan mode;

pulse frequency of the primary ion beam: 5 kHz (200 μsec./shot);

pulse width of the primary ion beam: approximately 1 ns; and

beam diameter of the primary ion beam: 5 μm.

<secondary ion: imaging was carried out by reconstructing the obtained data according to the application pattern of the primary ion beam>

detection mode for secondary ion: negative;

area for the measurement: 300 μm x 300 μm;

number of pixel in the secondary ion image: 128 x 128 pixels; and

number of integrating operation: 281.

**[0094]** Fig. 3 shows the analysis results via TOF-SIMS obtained by using $Ga^+$ and $Au_3^+$ according to the conditions described above. Fig. 3 includes the images for $PO_2^-$, $PO_3^-$,

$C_4H_4N_3^-$, $C_5H_5N_2O_2^-$, $C_5H_4N_5^-$ and $C_5H_4N_5O^-$, which are the typical secondary ions obtainable in the TOF-SIMS analysis for the DNA probe array containing ~~mixed~~ four mixed types of nucleic acid bases~~,~~ by using $Ga^+$ (shown in upper row) or by using $Au_3^+$ (shown in a lower row). Here, the description "mc" refers the maximum value in a pixel, and "tc" refers to the total count number in the whole 128 x 128 pixels. As seen in these images, employing $Au_3^+$ provides a nearly double-digit higher sensitivity for $PO_3^-$ ~~by nearly double digit,~~ and also provides a greater than a double-digit ~~much higher~~ sensitivity for the fragment ions derived from the four bases ~~by greater than double digit~~, ~~as~~ compared ~~with~~ to the case of employing $Ga^+$ (about 87-fold as reduced to the case of equivalent dosage, or about 20-fold as reduced to the case of equivalent measurement time (as 0.12-fold decrease in the pulse current, and 2-fold increase in the pulse cycle)). Thus, it was found that the use of the $Au_3^+$ gun for the TOF-SIMS analysis of the biochip was considerably advantageous.

**[0095]** While the present invention has been described with reference to what are presently considered to be the preferred embodiments, it is to be understood that the invention is not limited to the disclosed embodiments. ~~On~~ To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims. The scope of the following claims is to be accorded the broadest

interpretation so as to encompass all such modifications and equivalent structures and functions.

~~WHAT IS CLAIMED IS:~~

~~1. A method for acquiring information in relation to a device including a substrate and a plurality of materials disposed on a surface of said substrate from said surface of said device using time of flight secondary ion mass spectrometry, including at least the steps of:~~

~~irradiating pulsed primary ion beam on different positions of said surface of said biochip in a discontinuous pattern, and said primary ion beam having a spot size of smaller area than an area to be measured on said surface of said device;~~

~~conducting mass analysis of secondary ions via time of flight, said secondary ion being generated by irradiating said pulsed primary ion beam; and~~

~~reconstructing analyzed results obtained by conducting said mass analysis to form a two dimensional information on the basis of said pattern of said irradiating pulsed primary ion beam.~~

~~2. The method according to claim 1, wherein said discontinuous pattern is selected to be a two dimensionally random pattern.~~

~~3. The method according to claim 1, wherein said discontinuous pattern is selected to be a specifically programmed pattern.~~

~~4. The method according to claim 1, wherein an ion species of said primary ion beam is gold ion ($Au^+$, $Au_2^+$, $Au_3^+$).~~

~~5. The method according to claim 1, wherein the acquisition of information from the device surface is conducted by a combination of scanning of the primary ion beam and positional scanning of said substrate itself.~~

~~6. The method according to claim 1, wherein the device is a chip, on which biological-related materials are disposed.~~

~~7. The method according to claim 6, wherein said biological-related material is nucleic acid.~~

~~8. The method according to claim 7, wherein the nucleic acid is selected from the group consisting of DNA and RNA.~~

~~9. The method according to claim 8, wherein the DNA is selected from the group consisting of oligodeoxynucleotides, polydeoxynucleotides and cDNA (complementary DNA).~~

~~10. The method according to claim 6, wherein said biological-related material is PNA (peptide nucleic acid).~~

~~11. The method according to claim 6, wherein said biological-related material is protein.~~

~~12. The method according to claim 7, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation and ionization of phosphate backbone derived from nucleic acid.~~

~~13. The method according to claim 12, wherein the secondary ion species generated by said primary ion beam includes at least any one of $P^-$, $PO^-$, $PO_2^-$ and $PO_3^-$.~~

~~14. The method according to claim 8, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation and ionization of nucleic acid base.~~

~~15. The method according to claim 14, wherein the secondary ion species generated by said primary ion beam includes at least any one of $(adenine-H)^-$, $(thymine-H)^-$, $(guanine-H)^-$, $(cytosine-H)^-$ and (uracil-H).~~

~~16. The method according to claim 10, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation and ionization of peptide backbone.~~

~~17. The method according to claim 11, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation of amino acid residual group and species derived by the ionization of amino acid residual group.~~

~~18. The method according to claim 1, wherein said apparatus of time of flight secondary ion mass spectrometry for the use in the method is selected to be a reflectron type apparatus in which the measurement is carried out while said substrate is held in a condition of electrically grounded.~~

~~19. A method for analyzing components of a biological-related material disposed on a biochip in relation to the biochip, which includes a substrate, and a plurality of biological-related materials disposed on a surface of said substrate from said surface of said biochip using time of flight secondary ion mass spectrometry, including at least the steps of:~~

~~irradiating pulsed primary ion beam on said surface of said biochip in a discontinuous pattern, and said primary ion beam having a spot size of smaller area than an area to be measured on said surface of said biochip;~~

~~conducting mass-analysis of secondary ions via time of flight, said secondary ion being generated by irradiating~~

~~said pulsed primary ion beam;~~

~~reconstructing analyzed results obtained by conducting said mass-analysis to form a two-dimensional information on the basis of said pattern of said irradiating pulsed primary ion beam; and~~

~~conducting component analysis of the biological-related material of a necessary portion contained in the obtained two-dimensional image on the basis of the mass spectrum information of said necessary portion.~~

~~20. An apparatus for acquiring information in relation to a biochip including a substrate and a plurality of biological-related materials disposed on a surface of said substrate from said surface of said biochip using time of flight secondary ion mass spectrometry, including at least:~~

~~a means for irradiating pulsed primary ion beam on said surface of said biochip in a discontinuous pattern, said surface of said biochip having said biological-related material disposed thereon, and said primary ion beam having a spot size of smaller area than an area to be measured on said surface of said biochip;~~

~~a means for conducting mass-analysis of secondary ions via time of flight, said secondary ion being generated by irradiating said pulsed primary ion beam; and~~

~~a means for reconstructing analyzed results obtained by conducting said mass-analysis to form a two-dimensional information on the basis of said pattern of said irradiating~~

~~pulsed primary ion beam.~~

## ABSTRACT OF THE DISCLOSURE

A measurement method is provided, which enables to obtain a two-dimensional image with better quantitative-ability by suppressing the influence of the charge~~ing~~-up, when the two-dimensional secondary ion image is obtained for a biological ~~-related~~ material fixed on a substrate having a high resistivity by utilizing a TOF-SIMS method in a certain~~ly~~ wide area. ~~Two~~A two-dimensional image having considerably high positioning resolution-ability can be obtained by the procedure~~,~~ in which the pulsed primary ion beam is irradiated at a spot, and the pulse-wise spot-applications of the primary ion beam and the simultaneous detection of the secondary ion generated from the irradiated primary ion beam ~~are~~ proceed~~ed~~ along with a discontinuous scanning pattern, and eventually the results of these secondary ion measurements ~~results is~~ are reconstructed into a two-dimensional image in line with the aforementioned discontinuous scanning pattern.